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Brain tissue preparation and immunohistochemistry

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An abbreviated version of this protocol was published in Science Signaling in Sep 2020

Daily alcohol intake triggers aberrant synaptic pruning leading to synapse loss and anxiety-like behavior

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Detailed protocol

Brain tissue preparation and immunofluorescence for evaluating synaptic pruning by microglia

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Step-by-step:

- *Tissue preparation:*

1) Perfuse the mouse transcardially with ice-cold PBS (~10ml) and then with 4% PFA (~25-30ml) using an osmotic pump.

2) Dissect the brain tissue (with the olfactory bulb and cerebellum) and postfix it in 4% PFA in PBS, pH 7.2, for 12-18h.

3) Wash the tissue once with excess PBS.

4) Transfer the tissue to a 15 ml tube and cryoprotect the tissue using a sucrose gradient in a row — 15% sucrose then 30% sucrose (24h in each solution or until the tissue reaches the bottom of the tube).

5) After 24h, embed the tissue in optimal cutting temperature embedding medium (OCT – cat# 6769006; ThermoFisher) and store at -20ºC (short-term) or -80ºC (long-term).

6) Using a CM3050S Cryostat (Leica Biosystems) (chamber temperature at -24ºC and the object temperature at -18ºC), cut 30µm coronal sections of the desire brain regions.

7) Carefully collect the brain slices, non-sequentially, on Superfrost Plus slides (cat# J1800AMNZ; ThermoFisher).

➢ Tissue sections encompassing identical stereological regions from the brains of different mice should be collected on the same glass slides and stored at -20ºC.

8) Thaw slides with desired tissue sections for at least 1h before starting the immunostaining procedure.

- *Immunostaining:*

1) Hydrate the sections with PBS for 15 min.

2) Permeabilize the sections for 15 min.

3) Remove the permeabilization solution and wash sections in excess PBS for 10 min.

4) Carefully remove excess PBS and add the blocking solution for 1h at 22-25ºC.

5) Remove the blocking solution and incubate with primary antibodies (prepared in blocking solution) in a humidified chamber at 4ºC for 12-18h.

6) Remove primary antibodies and wash sections in excess PBS 3x10 min.

7) Remove PBS and incubate sections with secondary antibodies (1:600 AlexaFluor 488 [cat# A11008], 1:400 AlexaFluor 568 [cat# A11077], and 1:500 AlexaFluor

[illegible] 647 [cat# A21325]; all from ThermoFisher) in blocking solution for 2h at 22-25ºC.

➢ Depending on the microscope system available, antibodies` titration might require further optimizations.

8) Remove secondary antibodies and wash sections in excess PBS 3x10 min.

➢ If performing double or triple-labeling immunostaining, add the primary antibody followed by the adequate secondary antibody and repeat the process for each combination of primary/secondary antibodies. Never combine the primary antibodies in the same incubation step with a single master mix.

9) Coverslip the slides using Fluoroshield anti-fading reagent (cat# F6182; Sigma) and store at 4ºC.

10) Visualize the slides under a Leica TCS SP8 confocal microscope.

Solutions:

1. Permeabilization solution: 0.25% TritonX-100 in PBS
2. Blocking solution: 5% BSA (cat# MB04602; Nzytech) + 5% FBS (cat# 10270106; ThermoFisher) + 0.1% TritonX-100 in PBS

Tested primary antibodies:

Antibody	Dilution	Company	Catalogue Number
Iba1	1:500	Wako	019-19741;RRID:AB_839504
vGlut-1	1:1,000	Synaptic Systems	135 303; RRID:AB_887875
PSD95 -clone 6G6-1C9	1:600	Thermo Scientific	catalog no. MA1-045;RRID:AB_325399
CD68 - clone FA-11	1:400	Bio-Rad	MCA1957T; RRID:AB_2074849

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1. Socodato, R. , Summavielle, T. and Relvas, J. (2020). Brain tissue preparation and immunohistochemistry. Bio-protocol Preprint. bio-protocol.org/prep567.
2. Socodato, R., Henriques, J. F., Portugal, C. C., Almeida, T. O., Tedim-Moreira, J., Alves, R. L., Canedo, T., Silva, C., Magalhães, A., Summavielle, T. and Relvas, J. B.(2020). Daily alcohol intake triggers aberrant synaptic pruning leading to synapse loss and anxiety-like behavior . Science Signaling 13(650). DOI: 10.1126/scisignal.aba5754